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The receptive function of hypothalamic and brainstem centers to hormonal and nutrient signals affecting energy balance

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Abstract

The hypothalamic arcuate nucleus (ARC) and the area postrema (AP) represent targets for hormonal and metabolic signals involved in energy homeostasis, e.g. glucose, amylin, insulin, leptin, peptide YY (PYY), glucagon-like peptide 1 (GLP-1), and ghrelin. Orexigenic neuropeptide Y (NPY) expressing ARC neurons are activated by food deprivation and inhibited by feeding in a nutrient-dependent manner. PYY and leptin also reverse or prevent fasting-induced activation of the ARC. Interestingly, hypothalamic responses to fasting are blunted in different models of obesity (e.g. diet-induced or late-onset obesity). The AP also responds to feeding-related signals. The pancreatic hormone amylin acts via the AP to control energy intake. Amylin-sensitive AP neurons are also glucose-responsive. Furthermore diet-derived protein attenuates amylin responsiveness suggesting a modulation of AP sensitivity by macronutrient supply. This review gives an overview of the receptive function of the ARC and the AP to hormonal and nutritional stimuli involved in the control of energy balance and the possible implications in the context of obesity. Collectively, there is consistency between the neurophysiological actions of these stimuli and their effects on energy homeostasis under experimental conditions. However, surprisingly little progress has been made in the development of effective pharmacological approaches against obesity. A promising way to improve effectiveness involves combination treatments (e.g. amylin/leptin agonists). Hormonal alterations (e.g. GLP-1, PYY) are also considered to mediate body weight loss observed in obese patients receiving bariatric surgery. The effects of hormonal and nutritional signals and their interactions might hold the potential to develop poly-mechanistic therapeutic strategies against obesity.

Abbreviations:

a-MSH	a-melanocyte stimulating hormone
ADP	adenosine diphosphate
AgRP	agouti-related peptide
AMPK	adenosine monophosphate-activated protein kinase
AP	area postrema
APX	area postrema lesion
ARC	arcuate nucleus
ATP	adenosine triphosphate
CCK	cholecystokinin
cGMP	cyclic guanosine monophosphate
CPT1	carnitine palmitoyltransferase-1
CTR	calcitonin receptor
DIO	diet-induced obesity
DR	diet-resistant
FAS	fatty acid synthase
GHS-R	growth hormone secretagogue receptor
GLP-1	glucagon-like peptide-1
GLP-1R	glucagon-like peptide-1 receptor
LOO	late onset obesity
IPBN	lateral parabrachial nucleus
mTOR	mammalian target of rapamycin
NCD	non-caloric diet
NPY	neuropeptide tyrosine
NTS	nucleus of the solitary tract
OXM	oxyntomodulin
POMC	pro-opiomelanocortin
PVN	paraventricular nucleus
PYY	peptide tyrosine tyrosine
RAMP	receptor activity modifying protein
RYGB	Roux-en-Y gastric bypass
SDA	subdiaphragmatic deafferentation
STAT	signal transducer and activator of transcription

Introduction

Driven by the search for therapeutic treatment strategies against obesity and associated metabolic disorders, considerable knowledge has accumulated about the control mechanisms involved in the maintenance of energy balance. The redundancy of control mechanisms, the capacity of these systems to compensate for pharmacological effects, and the existence of obesity-related hormonal insensitivities make it difficult to achieve sufficient therapeutic efficiency for the reduction of body weight over longer periods of time. Evidently, at least under non-laboratory conditions, so-called non-homeostatic factors (hedonic properties and availability of food, food preferences, social factors, eating habits, etc.) and central reward mechanisms override homeostatic hormonal and metabolic signals that are considered to reflect and to control the body's energy status.

In contrast to the currently available pharmacological treatment options, bariatric surgery (e.g. Roux-en-Y gastric bypass, RYGB) is very effective in reducing body weight and improving glucose homeostasis in patients with type II diabetes mellitus [1, 2]. Apparently bariatric surgery alters gastrointestinal physiology in a way that changes nutrient-induced gastrointestinal hormone responses, which presumably contribute to these therapeutic effects. Hence, the signaling mechanisms of the gut-brain axis and the interaction between its nutritional and hormonal feedback signals are not only essential for our understanding of the body's energy homeostasis but also for the ultimate desire to develop pharmacological approaches against the obesity epidemic. Some of the hormones (e.g. GLP-1 and PYY) discussed in this review with regard to their central actions are presumed mediators of weight loss after bariatric surgery. While the exact mechanisms behind bariatric weight loss surgery and their potential to be targeted pharmacologically remain to be identified, there are other promising therapeutic approaches that are based on hormonal combination treatments. Both pre-clinical studies in obese rodents and clinical trials in humans have established a beneficial effect of combined treatment with the anorexigenic pancreatic hormone amylin with the fat-derived hormone leptin. Amylin treatment seems to reverse leptin insensitivity in obese subjects, in which amylin and leptin exert a synergistic action to reduce body weight [3, 4]. Brain circuits involved in the control of energy balance also function as fuel sensors responding to diet-derived macronutrients. Nutrients not only alter neuronal activity in feeding regulatory brain areas, they also modulate neuronal responsiveness to hormonal stimuli. Two important brain sites that are targeted by hormonal and nutrient signals involved in the control of energy balance are the hypothalamic arcuate nucleus (ARC) and area postrema (AP) of the brainstem. The current review focuses on the receptive function of the ARC and the AP to feeding-related signals and alterations of neuronal responsiveness that are associated with obesity. It is beyond the scope of this review to describe similar hormone and nutrient-dependent signaling mechanisms in other brain areas that also contribute to the overall control of energy homeostasis.

The hypothalamic arcuate nucleus

The ARC is considered as one of the most important hypothalamic target structures for blood-borne hormonal and metabolic factors involved in the regulation of food intake and energy homeostasis. The best-characterized hormone supposed to act via the ARC is the lipostatic and anorectic hormone leptin, a 16kDa protein that is secreted from white adipose tissue. Genetic deficiency of leptin or its receptor in rodents causes excessive food intake, body weight gain and an obese phenotype [5, 6]. Leptin acts via the ARC, which contains two functionally antagonistic populations of neurons with respect to the control of food intake. One population contains the orexigenic neuropeptides NPY and agouti gene-related peptide (AgRP) [7]. The other distinct population synthesizes the anorectic pro-opiomelanocortin (POMC) gene product α -melanocyte stimulating hormone (α -MSH) [8]. Leptin up-regulates POMC neurons while it inhibits NPY/AgRP neurons in the ARC [9, 10]. Similar to leptin, insulin fulfills the criteria of an adiposity signal because circulating insulin levels increase in relation to body adiposity. Furthermore, insulin has access to the brain and it influences food intake and body weight in a similar way as leptin via the ARC [11]. Catalyzed by the high importance of the ARC for body weight control and the failure of leptin therapy as an effective anti-obesity treatment because of leptin resistance [12] the scientific interest in other factors acting via the ARC and its downstream signaling pathways began to increase. The well-established concept that the ARC is targeted by the adiposity signals leptin and insulin has been extended by numerous studies demonstrating ARC-dependent actions of hormones, neuropeptides and metabolites that change dynamically according to the status of energy intake. Notably, leptin itself shows dynamic short-term changes as a function of food intake [13, 14].

Ghrelin

One of the hormones targeting the ARC is the orexigenic gastrointestinal hormone ghrelin [15]. Plasma levels of ghrelin are modulated by the nutritional state. During fasting and shortly before meals circulating ghrelin concentrations are elevated, while feeding or nutrient intake reduce blood ghrelin levels [16-18]. Various electrophysiological and immunohistological studies characterized ghrelin's effects on neuronal activity of ARC neurons that have been suggested to represent target cells for ghrelin in the brain [19-22]. Ghrelin binds to the growth hormone secretagogue receptor (GHS-R) that is highly abundant in the ARC [23]. The GHS-R is predominantly expressed in NPY neurons (94% co-localization) while it is found in only 8% of POMC neurons [24]. Ghrelin exerts opposite effects to leptin on the activity of ARC neurons. Ghrelin or GHS-R agonists excite leptin-inhibited NPY neurons via a direct postsynaptic effect [19, 25-27]. In addition to this action, ghrelin indirectly inhibits neurons of the lateral ARC, where POMC neurons are located [20, 28]. The

excitatory effect of ghrelin on ARC neurons and the NPYergic phenotype of these cells have been confirmed in vivo by immunohistochemical studies using c-Fos as a marker for neuronal activation [22, 29].

Whether ghrelin can be considered a physiological signal in the control energy homeostasis has not yet been confirmed based on the general criteria that have been suggested to define a physiological relevance [30]. Due to the weak consequences of ghrelin antagonism for feeding behavior or body weight phenotype and because of the ineffectiveness of physiological ghrelin doses to produce feeding or body weight effects, the physiological relevance of ghrelin has not yet been fully established [31]. Due to the leptin counter-regulatory action of ghrelin, antagonism of ghrelin signaling has been proposed as a therapeutic approach against obesity. However, the role of ghrelin in the pathogenesis of obesity is not clear. Ghrelin levels in obese humans are lower than under normal weight conditions [32] limiting the usefulness and success of ghrelin antagonism as an anti-obesity strategy [31]. Interestingly, weight loss in obese subjects seems to be associated with an increase in ghrelin levels suggesting that a blockade of ghrelin action might facilitate the body weight reducing effect of other therapeutic interventions [32]. Notably, there is cumulating evidence for a role of ghrelin in reward mechanisms that involve extra-hypothalamic pathways originating in the ventral tegmental area [33]. The potential of pharmacological ghrelin-dependent approaches with respect to this mechanism has not yet been sufficiently explored in clinical studies.

PYY/GLP-1

The two gut hormones PYY and GLP-1 act on ARC neurons. They have received special attention in the context of energy homeostasis and as potential targets for the treatment of overweight. Both hormones might contribute to weight loss after gastric bypass surgery because RYGB patients have increased GLP-1 and PYY levels and blockade of gastrointestinal hormone release (including GLP-1 and PYY) by the somatostatin analogue octreotide led to increased appetite [34-36]. A specific role for PYY is suggested by absence of weight loss in PYY knockout mice subjected to gastrointestinal bypass surgery [37].

PYY and PYY(3-36) are two biologically active peptides arising from endocrine L cells of the ileum and colon in response to food intake [38]. While PYY binds to several subtypes of Y receptors, PYY(3-36) exhibits relative specificity for the Y2 receptor [39]. Both PYY isoforms inhibit food intake after peripheral administration; but PYY(3-36) appears to be more potent possibly due to its specificity to the Y2 receptor that is decisive for the anorexigenic response [40, 41]. However, the minimal doses that were required to suppress food intake in these studies were supraphysiological when compared to meal-induced changes of total PYY blood levels. Therefore, the question whether PYY(3-36) acts as a physiological satiating signal is controversial. Direct site-

specific injections of PYY(3-36) identified the ARC as possible central target structure [42]. With respect to the electrophysiological effects of PYY(3-36) in the ARC, conflicting data exist in literature. In mice, PYY(3-36) has been demonstrated to indirectly (presynaptically) excite phenotypically identified POMC neurons [42], while another electrophysiological study demonstrated direct postsynaptic inhibitions in POMC neurons of mice [43]. Among others, the reasons for these seemingly contradictory findings have not yet been identified, but might be related to experimental and technical differences concerning the slice preparation and selection of cells [43]. In rats, full length PYY exerts direct inhibitory effects on ghrelin-excited ARC neurons (figure 1) suggesting that the NPYergic system is also affected by PYY signaling [44]. This is consistent with the demonstration that PYY(3-36) inhibits NPY release from ARC explants in addition to stimulating a α -MSH response [42].

Basal and postprandial PYY(3-36) levels are decreased in obese humans and rodents [45]. The absence of PYY(3-36) resistance in obese subjects is in favor of using PYY agonists as anti-obesity drugs. Chronic peripheral administration of PYY(3-36) decreases body weight in different species including rhesus macaques [42, 46, 47]. Whether PYY agonists can be therapeutically used in humans to reduce body weight awaits further evaluation. Interestingly, PYY seems to synergize with other hormones or hormone agonists affecting the control of energy balance and glucose homeostasis, including the GLP-1 agonist exendin-4 and the pancreatic hormone amylin [48, 49]. The neuronal correlates of these synergies are unknown.

Similar to PYY, GLP-1 is released in response to food intake from gastrointestinal L-cells [50, 51]. GLP-1 plays an important role in glucose homeostasis (incretin) and gastrointestinal function [52]. In addition to its peripheral expression, GLP-1 is a neuropeptide expressed in a discrete population of enteroceptive neurons located in the brainstem [53]. It binds to the GLP-1 receptor (GLP-1R) that is also activated by the agonist exendin-4, a peptide that has been isolated from the saliva of the lizard *Heloderma suspectum*. The truncated form of exendin(9-39) (also termed exendin-9) acts as a competitive antagonist at the GLP-1R. The ARC, and other hypothalamic and brainstem structures, including the paraventricular nucleus (PVN) and the AP, show high GLP-1R expression [54, 55]. Central and peripheral injection GLP-1R agonists decrease food intake in rodents [56-59]. Furthermore, numerous studies have demonstrated a GLP-1-mediated reduction in energy intake in humans [60]. Similar to ghrelin and PYY, it has not yet been confirmed that endogenous GLP-1 physiologically controls food intake.

The neuronal mechanisms mediating the action of GLP-1 on food intake have not yet been clearly identified, which might be due to the fact that different pathways seem to be activated depending on the experimental conditions, particularly the route of administration and the potency of the stimulus.

While subdiaphragmatic deafferentation (SDA) blocked the anorexigenic effect of hepatic portal vein GLP-1 infusion, SDA did not blunt the feeding suppressive effect of GLP-1 infused into the vena cava [61]. In contrast, the effect of the long-acting GLP-1 agonist exendin-4 was not blocked in SDA rats [62]. There is some evidence that the ARC represents a target site not only for GLP-1 but also for the structurally related hormone oxyntomodulin (OXM), which is co-secreted with GLP-1 and exerts an anorexigenic effect via the GLP-1R after central injection [63, 64]. GLP-1R activation by intra-arcuate injection of OXM decreased food intake and the OXM-mediated suppression of intraperitoneal OXM administration was blocked by injection of the GLP-1R antagonist exendin(9-39) [58]. Another study did not observe a feeding suppressive effect after intra-arcuate GLP-1 injection, but hepatic glucose production was reduced under these conditions [65]. However, GLP-1 consistently reduced feeding after site-specific injection into the PVN [65, 66].

The effects of GLP-1 and OXM on neuronal activity in the ARC are similar. In rats, both hormones induced excitatory responses in ghrelin-inhibited cells (see example in figure 2) suggesting a stimulation of POMC signaling, which is consistent with the high expression of the GLP-1R in POMC neurons [65]. GLP-1 and OXM induced heterogeneous excitatory and inhibitory effects in ghrelin-excited ARC neurons, but the functional and neurochemical phenotype of these cells has not yet been identified. The absence of GLP-1R expression in NPY neurons seems to exclude the NPY system as a primary target for GLP-1 in the ARC. Despite the existing *in vivo* and *in vitro* evidence for a potential role of ARC-dependent GLP-1 signaling in energy and glucose homeostasis further support for a physiological role of endogenous GLP-1R ligands acting via hypothalamic circuits needs to be established. Although beneficial effects on body weight have been reported for anti-diabetic GLP-1 agonists [67-69], moderate effectiveness and undesired side effects limit the potential of these drugs at least as a mono-therapy against obesity.

Feeding and nutrient-related effects on neuronal function

Numerous studies have confirmed the reciprocal regulation of NPY and POMC gene expression in response to alterations in energy intake [70]. Fasting not only leads to alterations in neuropeptide expression but also to changes in neuronal activity. Food deprivation for 24h elicits an activation of ARC neurons in mice as reflected by an increase in c-Fos expression [71-73]. Refeeding of 12h food-deprived mice completely reverses the fasting-induced ARC activation indicating that feeding-related signals exert a strong inhibitory effect on ARC neurons that are activated under negative energy balance [44, 74]. Hence, ARC neurons appear to respond to negative and positive changes in energy intake. As demonstrated by phenotyping studies, fasting-induced ARC activation specifically occurs in NPY neurons, but in not POMC neurons under these conditions [74, 75].

While this observation suggests increased hypothalamic NPY signaling as a result of fasting, it does not exclude a parallel inhibition of POMC neurons in the ARC. The induction of a fasting-induced ARC activation has also been demonstrated in rats, although considerably longer periods of food deprivation (3 days) are typically required for an immunohistochemically detectable response in this species, at least when c-Fos is used as a marker. Interestingly, there seems to be a shift of neuronal activation from the NPY system in the fasted state to the melanocortin system following refeeding [76, 77]. This response is consistent with the function of the NPY system to promote energy intake under negative energy balance and with the counter-regulatory function of the POMC system to limit energy intake.

Feeding of palatable non-caloric diet (NCD; vanilla-flavored cellulose) with or without selective nutrient supplementation has proven to be a useful approach to explore the role of diet-derived nutrients in feeding-related modulation of neuronal ARC activity. Mice receiving NCD for 12h instead of standard chow exhibit a strong c-Fos expression in the ARC that is only slightly lower than in 12h fasted animals [74]. The same is true for fasted mice that were refed with NCD. Therefore, nutrient-independent stimuli associated with feeding activity (e.g. gustatory, oropharyngeal cues, gastric distension) appear to exert a minor but significant influence on the ARC because they only weakly counteract the stimulation of ARC activation triggered by short term caloric deprivation. When refeeding with NCD was selectively supplemented with each of the different macronutrients (carbohydrates, protein or fat), a stronger reversal of fasting-induced ARC activation was observed than that following refeeding with non-supplemented NCD [74]. When the intake of each nutrient was matched to specific nutrient intake of chow-fed animals, the carbohydrates were more effective than protein and fat. However, increasing the protein content in the diet or refeeding with pure fat (lard) resulted in a similar reversal of fasting-induced c-Fos expression as observed for refeeding with carbohydrate. Therefore, the relative contribution of each macronutrient to the inhibition of ARC activity in this experimental setting appeared to depend on the amount of intake. Whether isocaloric intakes of the different macronutrients are equipotent to reverse fasting-induced ARC activation has not yet been investigated.

The mechanism underlying the fasting and feeding-related effects on neuronal activity in the ARC are likely to involve direct effects of metabolites but also indirect actions mediated by gastrointestinal hormones. ARC neurons have been shown to respond to glucose in excitatory and inhibitory ways [78]. Phenotypically identified NPY neurons of mice and rats decrease their firing rate with increasing glucose concentrations (glucose-inhibited cells) [79, 80]. Conversely, POMC neurons have been demonstrated to be glucose-excited because they decrease their discharge rate when the extracellular glucose concentration is decreased [81, 82]. The intracellular mechanisms transducing changes in the glucose concentration into altered neuronal activity are well understood

for glucose-excited neurons, while the molecular correlates of inhibitory glucose signaling are less clear. Glucose sensitivity in glucose-excited neurons has obvious parallels to glucose responsiveness of pancreatic beta-cells, in which glucose metabolism leads to increased ATP:ADP ratios. As a consequence ATP induces a closure of the Kir6.2 pore-forming subunit of the ATP-sensitive potassium channel, leading to a depolarization of the cell. Genetic manipulations that cause a loss of function of Kir6.2 subunit are associated with a loss of glucose responsiveness in hypothalamic neurons, including POMC neurons of the ARC [82, 83]. Although the ATP-sensitive potassium channel is required for the glucose sensitivity of glucose-excited neurons, it is also expressed in glucose-insensitive cells suggesting that other molecular transducers are decisive for neuronal glucose responsiveness. One of these transducers is the enzyme glucokinase, the rate-limiting enzyme for glucose metabolism in glucose sensitive neurons [84]. Another enzyme that seems to function as a master switch for intracellular metabolic pathways including glucose metabolism is the enzyme adenosine monophosphate-activated protein kinase (AMPK). AMPK responds to changes in the ADP:ATP ratio and induces counter-regulatory metabolic responses via a variety of downstream targets [85]. Hypothalamic AMPK activity in the ARC and other hypothalamic structures is not only inhibited by leptin and insulin, but also by refeeding in fasted mice and by intraperitoneal and intracerebroventricular glucose administration [86]. Genetic manipulation that results in constitutive hypothalamic AMPK activity was associated increased body weight gain, while reduced AMPK activity resulted in reduced body weight. These effects were partly attributed to changes in food intake [86]. Genetic disruption of AMPK in POMC and NPY neurons was paralleled by a loss of glucose responsiveness of these cells in the ARC supporting of a role of AMPK in glucose sensing [81]. In line with the well-established glucose sensitivity of ARC neurons, an increase in circulating glucose after intraperitoneal glucose administration reversed the fasting-induced ARC activation to a similar degree as refeeding [87]. These studies do not exclude indirect hormonal or vagal afferent signaling mechanisms that might be engaged in parallel to a direct action of glucose on ARC neurons.

Not only glucose but also hypothalamic fatty acid signaling contributes to the control of energy balance and glucose homeostasis because intracerebroventricular infusion of oleic acid inhibits food intake and hepatic glucose production [88, 89]. Besides other hypothalamic structures, the ARC has been identified as a fatty acid responsive area [90]. Lipid sensitivity has been demonstrated for POMC neurons that were inhibited by oleic acid [91]. In analogy to the importance of intracellular glucose metabolism, intracellular lipid metabolism has been linked to altered neuronal function and to changes in food intake and body weight. Pharmacological blockade fatty acid synthase (FAS) pathway by central or peripheral injection of the FAS inhibitor C75 inhibited feeding and reduced body weight in mice [92]. Among other hypothalamic sites, FAS is expressed in NPY neurons of

the ARC [93]. Peripherally applied C75 prevented the fasting induced c-Fos expression in the medial ARC and in hypothalamic downstream targets of the ARC supporting a role of lipid signaling in fasting-induced ARC activation [94]. Different intracellular mechanisms have been proposed to mediate the effects of FAS inhibition on energy homeostasis. These mechanisms include an increase in the FAS substrate malonyl CoA, which represents an intracellular metabolic signal inhibiting food intake and body weight. This view is consistent with the development of obesity and increased food intake when malonyl CoA degradation in the mediobasal hypothalamus is enhanced by overexpression of the enzyme malonyl CoA decarboxylase [95]. A regulatory function for cellular lipid sensing has also been postulated for the enzyme carnitine palmitoyltransferase-1 (CPT1) that controls long chain fatty acid transport into the mitochondria. Pharmacological or genetic inhibition of hypothalamic CPT1 reduced food intake and glucose production [96]. Despite the cumulating evidence for a role of hypothalamic lipid sensing further confirmation is required for the physiological relevance of these processes in the context of feeding-related or metabolically induced changes in circulating mediators involved in lipid signaling.

Similar to lipid signaling a role of hypothalamic amino acid signaling in the control of food intake and body weight has emerged. The serine-threonine kinase mammalian target of rapamycin (mTOR) functions as nutrient sensor implicated in cell growth and proliferation [97]. Particularly branched amino acids such as leucine activate the mTOR pathway. Components of the mTOR pathway have been detected in 90% and 45% of ARC NPY and POMC neurons, respectively [98]. In analogy to the changes of neuronal activity in the ARC as a result of fasting and refeeding, activity of the mTOR pathway in the ARC is down-regulated in the fasted state while it increases in response to refeeding. Both, intracerebroventricular and site-specific injection of leucine into the mediobasal hypothalamus inhibited food intake [98, 99]. POMC neurons of the ARC increase their electrical activity in response to stimulation with leucine, which is consistent with in vivo studies demonstrating a melanocortin receptor involvement in the anorexigenic effect of mediobasal hypothalamic leucine injection [99].

Fasting and the ingestion of each of the single macronutrients lead to hormonal responses adjusting energy homeostasis and gastrointestinal function to the metabolic and digestive requirements of the body. Some attempts have been made to explore the involvement of hormones in the feeding-related changes in ARC activity. Based on the increase in ghrelin levels under fasting conditions ghrelin might contribute to the fasting-induced ARC activation in mice. In rats, neutralization of ghrelin by a site-specific injection of anti-ghrelin immunoglobulins into the ARC attenuated food intake in fasted animals during refeeding [100]. An alternative approach to neutralize ghrelin is the use of an L-RNA-based hormone antagonist, a so-called Spiegelmer, that specifically binds and inactivates the bioactive form of ghrelin [101, 102]. Due to its long-lasting action a single

intravenous infusion of the anti-ghrelin Spiegelmer NOX-B11-3 blocked the c-Fos response in the ARC of ad libitum fed mice induced by exogenous ghrelin injected peripherally 12h after administration of the antagonist [21]. In the same study, ghrelin antagonism did not significantly attenuate the fasting-induced c-Fos response in the ARC suggesting that endogenous ghrelin is not necessary for the activation of the ARC under these experimental conditions.

Among the feeding-related hormones that are likely to act via the ARC ghrelin is the only one that increases during fasting. Not only increases but also decreases in hormone levels are likely to reflect alterations in energy homeostasis and metabolism. One experimental approach to investigate this consists in the compensation of decreased hormone levels by exogenous administration of the respective hormone. Superimposed to the well-described differences in leptin levels related to body adiposity, leptin plasma levels decrease considerably (5-fold) in fasted vs. ad libitum fed lean mice [74]. Preventing the fasting-induced decrease in leptin by repeated low dose leptin injections during the fasting period markedly attenuated the c-Fos expression of in the ARC of food-deprived mice (figure 4) [103]. Despite the limitation of this approach to precisely mimic physiological leptin levels by exogenous leptin administration, this finding supports a contribution of decreased leptin signaling to fasting-induced ARC activation. Studies in leptin deficient ob/ob mice seem to complement these findings because fasting-induced ARC activation is exaggerated in ob/ob mice compared to wild-type littermates [103]. Together these studies underscore that leptin signaling has an important impact on the ARC in the context of feeding-related modulation of ARC neurons.

Using the fasting/refeeding paradigm the possible involvement of other hormones in feeding-related changes of ARC activity have been studied. Similar to the ineffectiveness of an acute leptin treatment to reverse fasting-induced ARC activation, insulin did not attenuate c-Fos expression in fasted animals regardless of whether a glucose-lowering dose of insulin was used or a dose that did not produce a reduction in blood glucose [74]. Besides insulin the anorexigenic hormones PYY, amylin and cholecystokinin (CCK) were tested for their ability to reverse fasting-induced c-Fos expression in the ARC. Only PYY, but not amylin or CCK attenuated the stimulation of ARC neurons in food-deprived mice [74, 104]. The lack of effectiveness was probably not due to ineffective doses because in the same animals both amylin and CCK elicited c-Fos expression in the AP and the NTS, respectively. Since amylin and CCK primarily act via the brainstem (see below) and because the ARC is considered as a target site for PYY, these finding are consistent with the presumed central signaling mechanisms for these hormones. Despite the failure of CCK and amylin to reverse fasting-induced ARC activation, at least CCK partially prevented ghrelin-induced c-Fos expression in the ARC in ad libitum fed mice [74] indicating some CCK-dependent inhibitory input to the ARC. Collectively, these findings suggest diverse roles for the investigated hormones in

feeding-related changes of neuronal function in the ARC. However, the use of knockout models or specific hormone antagonists is required to confirm the relevance of endogenous hormone actions.

Altered neuronal ARC responses in obese rodent models

Obesity is associated with hormonal and metabolic perturbations, including hyperleptinemia, hyperinsulinemia, leptin and insulin resistance, dyslipidemia and hyperglycemia and others [12, 105, 106]. In rodent models of diet-induced obesity (DIO) neuronal responsiveness of ARC neurons to leptin is disrupted. DIO mice show a reduced phosphorylation of the signal transducer and activator of transcription 3 (STAT3), a marker for leptin receptor activation [107, 108]. Furthermore, the leptin-dependent release of NPY and α -MSH [109] is decreased. As recently demonstrated, obesity also seems to be associated with ghrelin resistance in the ARC because peripheral or central ghrelin injection did not induce food intake and ARC activation in DIO mice [110]. Moreover, ghrelin failed to induce the release of NPY and AgRP in hypothalamic explants from DIO mice in this study. Not only hormonal resistance but also impaired responsiveness of the ARC to metabolic stimuli has been described [111].

It has been demonstrated in different obesity models that obese mice show blunted neuronal responses of the ARC to alterations in energy intake. Age-related obese (late onset obesity, LOO) and DIO mice show completely absent or a strongly attenuated fasting-induced activation of NPY neurons in the ARC depending on the duration of food deprivation (12h or 24h, respectively). The blunted responses in these animals were not due to age per se or due to high-fat diet feeding because age-matched controls and diet-resistant lean controls (DR) showed intense fasting-induced c-Fos responses in the ARC [103, 112]. The absence of fasting-induced ARC activation was associated with the absence of refeeding hyperphagia that only occurred in lean mice after fasting but not in LOO mice. The blunted ARC activation in these hyperleptinemic obesity models contrasts with the exaggerated fasting response in the ARC of obese leptin deficient ob/ob mice [112]. Measurements of metabolic (blood glucose and lipids) and hormonal parameters (insulin, ghrelin, leptin) were conducted in fasted and ad libitum fed obese mice and in respective lean controls [103, 112]. Among these parameters, the only factor that consistently predicted the altered neuronal responses across the different obesity models was leptin because neuronal ARC activation is enhanced under leptin deficiency, prevented by exogenous leptin and blunted under hyperleptinemia. However, the idea that obesity-related hyperleptinemia prevents the response of the ARC to fasting is challenged by well-described leptin resistance in hyperleptinemic obesity. Factors that limit central effectiveness of circulating leptin involve reduced transport across the blood-brain barrier, increased intracellular inhibition of leptin-signaling and reduced leptin receptor expression. Each of these factors is affected by feeding in a way that promotes leptin

responsiveness under fasting conditions. Fasting increases the rate of leptin transport into the brain [113, 114], reduces intracellular leptin-antagonistic signaling [115, 116] and increases leptin receptor expression [117] and leptin binding to the receptor [118]. Hence, leptin responsiveness is dynamic and linked to the feeding status. In line with this assumption, fasting not only increases the leptin-induced STAT3 phosphorylation in lean mice but also in LOO mice that did not show a significant pSTAT3 response in the ad libitum fed state [103]. Consequently, the combination of increased leptin levels and increased leptin responsiveness might prevent the activation of ARC neurons under negative energy balance. Together these findings suggest that dynamic changes in leptin sensitivity under hyperleptinemia might interfere with the receptive function of the ARC for stimuli reflecting short-term excursions in energy intake.

The area postrema

The AP is a sensory circumventricular organ located in the dorsal vagal complex of the brainstem. Due to the lack of a functional blood-brain barrier AP neurons can be reached by humoral factors including peptide hormones, that do not passively cross the blood-brain barrier in other areas of the brain. The AP is neuronally interconnected with structures of the visceral enteroceptive neuroaxis including the nucleus of the solitary tract (NTS) and the lateral parabrachial nucleus (LPBN) [119]. Among other neuronal efferents the AP also projects to the dorsal motor nucleus of the vagus that controls visceral motor functions. The AP plays an important protective role as a so-called chemoreceptor trigger zone that mediates aversive and emetic responses induced by noxious or toxic stimuli. This function is for example reflected by the absence of lithium chloride induced taste aversion after ablation of the AP lesioned in rats [120].

Observations from AP lesion studies suggested an AP involvement in the control of food intake and body weight. AP-lesioned (APX) rats are hypophagic and show lower body weight compared to sham-lesions animals, although they over-consume highly palatable food [121, 122]. Furthermore, AP lesion leads to loss of hypoglycemic or glucoprivic feeding responses induced by insulin, 2-deoxy-glucose or 5-thio-glucose [121, 123], suggesting a role of the AP in central nervous glucose sensing.

AP-mediated effects of amylin on food intake

The AP has been identified as a target structure for the anorexigenic hormone amylin, which is widely accepted to contribute to the physiological control of food intake. Amylin is co-secreted with insulin from pancreatic beta cells in response to meal-related stimuli [124]. Amylin reduces food intake at near physiological doses via a reduction of meal size [125-127]. Amylin antagonism not only blocks the anorexigenic effect of exogenous amylin but also increases food intake,

supporting a physiological role of endogenous amylin in energy intake [128]. Numerous studies provided evidence that the AP mediates amylin's suppressive effect on food intake. While vagal afferents are not required for amylin's anorexigenic action [129], AP lesion blocks feeding inhibitory action of amylin [130-132].

The effects of amylin on neuronal function in the AP and the transmission of amylin signaling to other brain structures involved in the control of energy homeostasis are well established. The amylin receptor consists of a functional complex of the calcitonin receptor (CTR) and so-called receptor activity modifying proteins (RAMP1 and RAMP3) [133]. The CTR is densely expressed in the AP [134], where also RAMP3 gene expression has been described [135]. In electrophysiological studies, amylin exerted strong excitatory effects in 44% of the recorded spontaneously active AP neurons [136]. These effects were blocked by the amylin receptor antagonist AC187 and appeared to be mediated by the intracellular second messenger cyclic guanosine monophosphate (cGMP). The excitatory action of amylin on AP neurons is in line with studies demonstrating an amylin-induced c-Fos expression in the AP [136, 137]. Not only exogenous but also feeding-related endogenous amylin activates AP neurons because refeeding of fasted rats triggered a c-Fos response in the AP that was attenuated by amylin antagonism [138]. About 50% of neurons showing amylin-induced c-Fos are noradrenergic and specific neurotoxic lesion of these cells blunted amylin's feeding inhibitory action [139].

Interaction of amylin and nutrient signaling

Glucose

In support of the abovementioned evidence for a role of the AP as central glucose sensor, electrophysiological studies identified glucose-responsive neurons in the AP [140]. These studies were extended by the demonstration that amylin sensitive neurons in the AP are specifically excited by glucose (figure 5), whereas amylin insensitive neurons are unresponsive to changes in the ambient glucose concentration [141]. Based on the importance of amylin sensitive AP neurons for the physiological control of feeding behavior these findings further support a role of the AP in the metabolic control of food intake. The AP is not the only glucose sensitive structure in the brainstem, but it is located in a brainstem area where local injection of 5-thio-glucose induced the strongest glucoprivic feeding responses [142].

The glucose sensitivity of the AP has also been postulated as protective mechanism against excursions in blood glucose values. Hypoglycemia induces a counter-regulatory increase in the rate of gastric emptying [143] that compensates the decreased blood glucose via increased gastrointestinal nutrient absorption. This mechanism interferes with hormonal and pharmacological effects that delay gastric emptying because glucose appears to be a permissive factor. Amylin

potently inhibits gastric emptying via an AP-dependent action, which is overridden by hypoglycemia [144]. Our group recently demonstrated a similar permissive effect of glucose for amylin's feeding suppressive effect because amylin failed to significantly inhibit feeding under hypoglycemic conditions (Neuner-Boyle, C.; unpublished observations). The inhibitory effect of the amylin agonist pramlintide on gastric emptying is an important therapeutic mechanism to lower postprandial glucose levels in diabetic patients. Since treatment-induced hypoglycemia is a severe risk in diabetic patients, the permissive effect of glucose on gastric emptying is considered as a fail-safe mechanism that overrides the anti-diabetic action of amylin agonism under hypoglycemic conditions (see [145, 146] for review).

Protein

Amylin's satiating effect and amylin-induced AP activation are modulated by diet-derived macronutrients, in particular by protein. An influence of diet-derived nutrients on amylin signaling was inferred from the observation that amylin-induced activation of the AP is stronger in fasted vs. ad libitum fed rats and also in rats that received nutrient-deficient NCD [147]. Selective supplementation of NCD with protein but not with glucose or fat strongly attenuated the amylin-induced AP activation suggesting that protein counteracts anorexigenic amylin signaling. In light of the well-established satiating action of protein this assumption appeared unexpected, but it was confirmed by feeding studies in which amylin failed to inhibit protein supplemented NCD (figure 6) [147]. The inhibitory effect of diet-derived protein on amylin action has been corroborated by immunohistological and feeding studies using isocaloric diets of different protein content. Amylin more potently decreased intake of 1% and 8% protein chow than standard chow containing 18% protein. Similarly amylin-induced c-Fos expression was higher in rats receiving less protein [148]. These effects were not secondary to altered caloric intake that was similar across the experimental groups. While blood glucose levels were also similar, blood amino acid levels decreased in rats fed low protein diet suggesting that circulating amino acids might contribute the protein-dependent attenuation of amylin-induced AP activation. Pre-treatment of rats with an intraperitoneal administration of a mixed amino acid solution also attenuated amylin's c-Fos response in the AP, which supports this hypothesis [148]. However, the exact neuronal mechanism underlying the protein-dependent modulation of amylin signaling remains to be identified. The relationship between reduced amylin-responsiveness and protein intake might bear both physiological and therapeutic implications. Amylin's function in the control of nutrient and energy intake might be adapted to the nutrient composition of the diet. At least under undisturbed energy homeostasis protein is metabolically less important than carbohydrate or fat, which might suggest that amylin's contribution to the overall control of energy intake is stronger when the intake of protein relative to

glucose or fat is low. Another mutually not exclusive implication might consist in an AP-sensitizing effect produced by short term reduction of food intake or food availability in a protein-dependent manner, pre-adapting AP responsiveness to a higher level in the pre-prandial state before meal onset. In addition to the possible physiological relevance, the effectiveness of pharmacological approaches based on amylin agonism might be influenced in a nutrient-dependent way.

Fat

There is not much experimental evidence for a role of the AP in lipid sensing. Although lipoprivic feeding induced by the fatty acid oxidation inhibitor mercaptoacetate is blunted in rats after AP/NTS lesion, this effect has been attributed to the destruction of vagal terminal fields in the NTS [149]. Accordingly, lipoprivic feeding is also blocked in vagotomized rats, confirming a mediation of vagal afferent signals [149]. Similarly, c-Fos expression in the AP/NTS region after intraduodenal lipid infusion is attenuated by capsaicin-induced destruction of vagal afferents and by CCK receptor antagonism. The latter finding indicates an indirect effect of intestinal lipid sensing via CCK-mediated activation the vagal afferents signaling [150]. It is reasonable to assume that amylin signaling and vagally transmitted lipid/CCK dependent signals converge at the level of the AP/NTS region. Furthermore, the existence of a satiogenic synergy between amylin and CCK [151] might imply that lipid dependent signals indirectly enhance amylin's feeding suppressive effect via a CCK-dependent action. Unlike the development of hormonal resistance to leptin and insulin as a consequence of prolonged high fat diet feeding, neither short-term nor long-term high-fat diet feeding leads to amylin resistance at least when amylin's inhibitory action on food intake is used as an index of amylin responsiveness [152].

Hormonal interactions involving AP and brainstem mechanisms

The dorsal vagal complex is a site of convergence for vagal and blood-borne signals controlling food intake. Brainstem structures including amylin sensitive sites are reciprocally interconnected with hypothalamic and extra-hypothalamic areas involved in energy homeostasis [153]. For some hormones known to act via the brainstem synergistic effects have been described, although the existence of a synergy is not always based on equal criteria in the literature. Synergy is often inferred from the observation that the effect of two combined stimuli is higher than the sum of the effects when each stimulus is applied alone. Sophisticated statistical models (e.g. isobolographic analysis) have been used to detect and describe synergistic actions. Several synergistic effects have been demonstrated for amylin in combination with other hormones [154]. In addition to the synergy between amylin and CCK mentioned above, there is a synergistic effect of amylin and leptin to reduce body weight in obese and lean subjects [3, 4]. Several neuronal mechanisms have been

associated with the synergy between amylin and leptin. Amylin seems to enhance neuronal leptin responsiveness as reflected by the reversal of the blunted pSTAT3 response to leptin in the ventromedial hypothalamus. Amylin affects leptin signaling in the brainstem because amylin knockout mice tend to show reduced leptin-induced STAT3 phosphorylation in the NTS, and pre-treating leptin-resistant DIO rats with amylin increased leptin-dependent pSTAT3 signaling in the AP [3, 4].

Anorexigenic synergy has also been reported for amylin and PYY(3-36), whereas body weight reduction was additive [49]. The neuronal correlate of this anorexigenic synergy has not yet been identified. While the AP was not required for PYY(3-36)-induced suppression of feeding in one study [155, 156], another study suggested an involvement of the AP in the PYY-dependent effects on gastric acid secretion [157]. Binding sites for PYY of the Y receptor family are abundant in the AP [158-160]. Interestingly, a dose of full length PYY that did not trigger a c-Fos response in the AP alone, increased amylin-induced c-Fos expression in the AP [104] suggesting a possible interaction between amylin and PYY in the AP.

A recent study conducted in non-human primates demonstrated anorexigenic synergy between the potent amylin receptor agonist salmon calcitonin and the GLP-1R agonist exendin-4 [161]. The mechanism underlying this synergy also remains to be identified. There is evidence that GLP-1 inhibits feeding via an AP-dependent effect after hepatic portal vein infusion [162]. However, the anorexigenic effect of exendin-4 is not blocked in APX rats [163]. Although amylin and GLP-1 activate AP neurons, these hormones seem to have different target cells in the AP. Similar to the aversive stimulus lithium chloride, GLP-1 and exendin-4 almost exclusively activate AP neurons that do not express the CTR, which is considered as a marker for the primary target cells for amylin in the AP [148]. The functional implication of this dissociation has not yet been specifically investigated. GLP-1R-mediated activation of the AP has been linked to the induction of aversion [164]. Therefore it is particularly important to exclude aversive mechanisms in approaches based on GLP-1R activation.

Conclusions

Extensive evidence has accumulated demonstrating that the ARC and the AP are responsive to hormonal and nutrient-related signals involved in the homeostatic feedback control of energy homeostasis. Overall, the neurophysiological effects that have been described by a multitude of different *in vivo* and *in vitro* approaches are consistent with the effects of these signals on food intake, body weight or metabolism. However, for many of the discussed mechanisms the physiological relevance remains to be confirmed. Even when physiological relevance is established (e.g. amylin) or postulated (ghrelin, PYY, GLP-1) the predictive value of these physiological

mechanisms for the effectiveness of mono-therapeutic anti-obesity approaches targeting these mechanisms appears to be weak. Nevertheless, the impressive effectiveness of bariatric surgery suggests the existence of signaling mechanisms that potently reduce body weight and improve metabolic disorders without being compensated by the body. Obviously, the interaction between the different feedback signals has been underestimated, which is reflected by the promising improvements in the therapeutic outcome of combination treatments. The concept of the homeostatic feedback control of energy homeostasis needs to be integrated into the increasing knowledge about the non-homeostatic controls affecting energy balance.

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The author does not declare conflicts of interests.

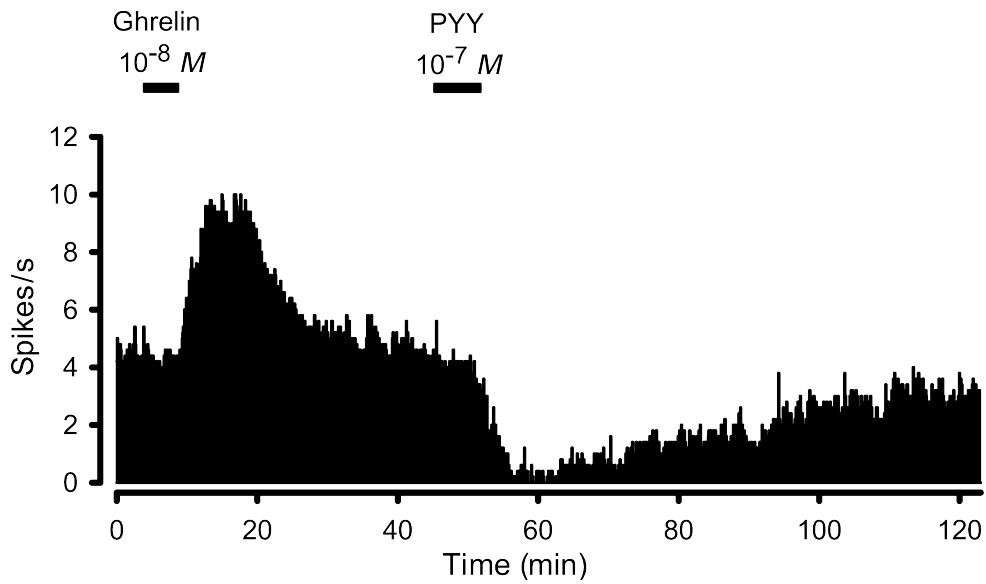


Figure 1: Continuous rate meter recording of a spontaneously active neuron from the medial arcuate nucleus of the rat. Consecutive superfusions of ghrelin and the anorectic hormone peptide Y (PYY) at the indicated times caused opposite effects on neuronal activity. While ghrelin induced a strong excitatory response, PYY effectively decreased the discharge rate. Reproduced with permission (published in Riediger T. et al, Neuroendocrinology 2004;79:317-326).

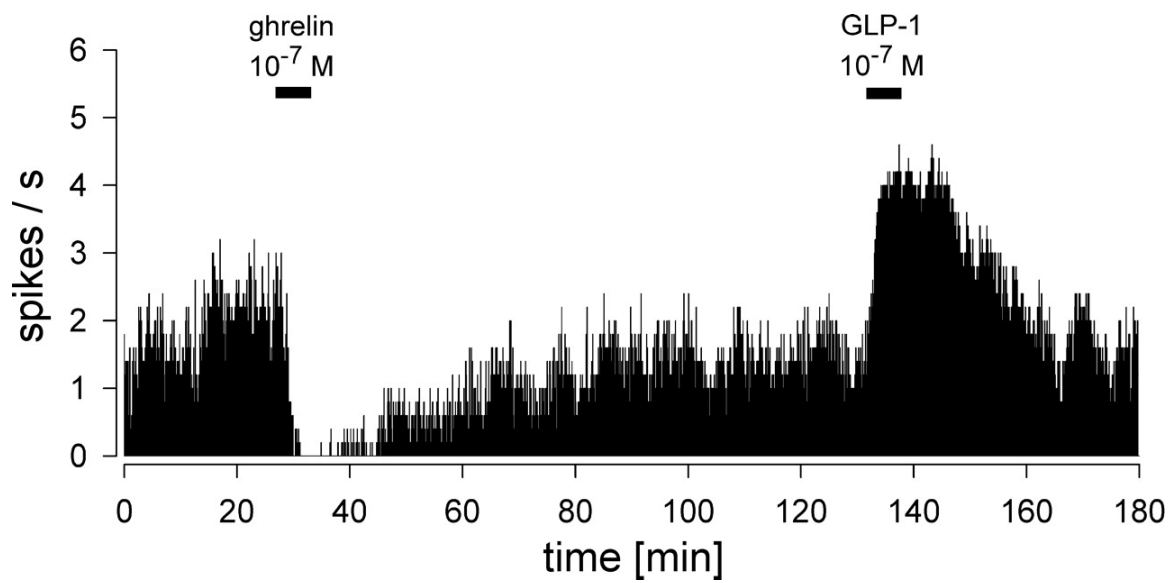


Figure 2: Representative recording showing the excitatory effect of glucagon-like peptide-1 (GLP-1) on a ghrelin-inhibited neuron of the arcuate nucleus. Published in Riediger T. et al., Am J Physiol Regul Integr Comp Physiol 2010;298:R1061-R1067.

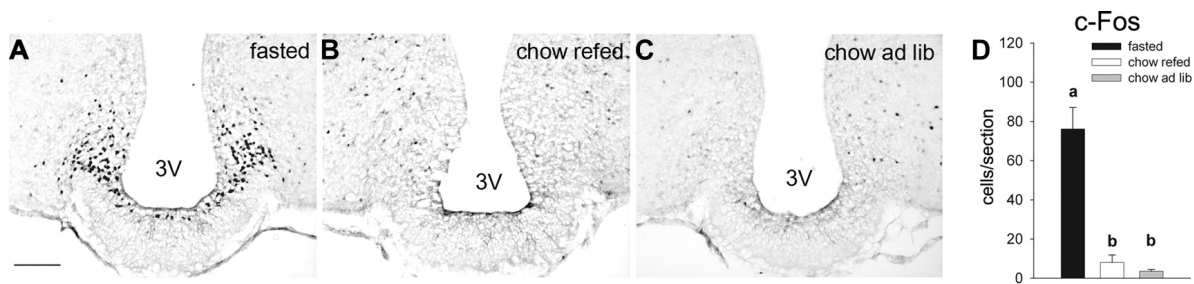


Figure 3: Refeeding with chow completely reversed the fasting-induced c-Fos expression in the arcuate nucleus (ARC). Representative ARC sections immunostained for c-Fos of 14-h fasted (A), chow-refed (B) and ad libitum chow-fed (C) mice. Bar charts show the quantitative results of c-Fos expression (D). a,b Different letters indicate significant differences between groups ($P < 0.05$). 3V: 3rd ventricle. Scale bar: 100 μm . Published in Becskei C. et al., Am J Physiol Regul Integr Comp Physiol 2009;297:R100-R110.

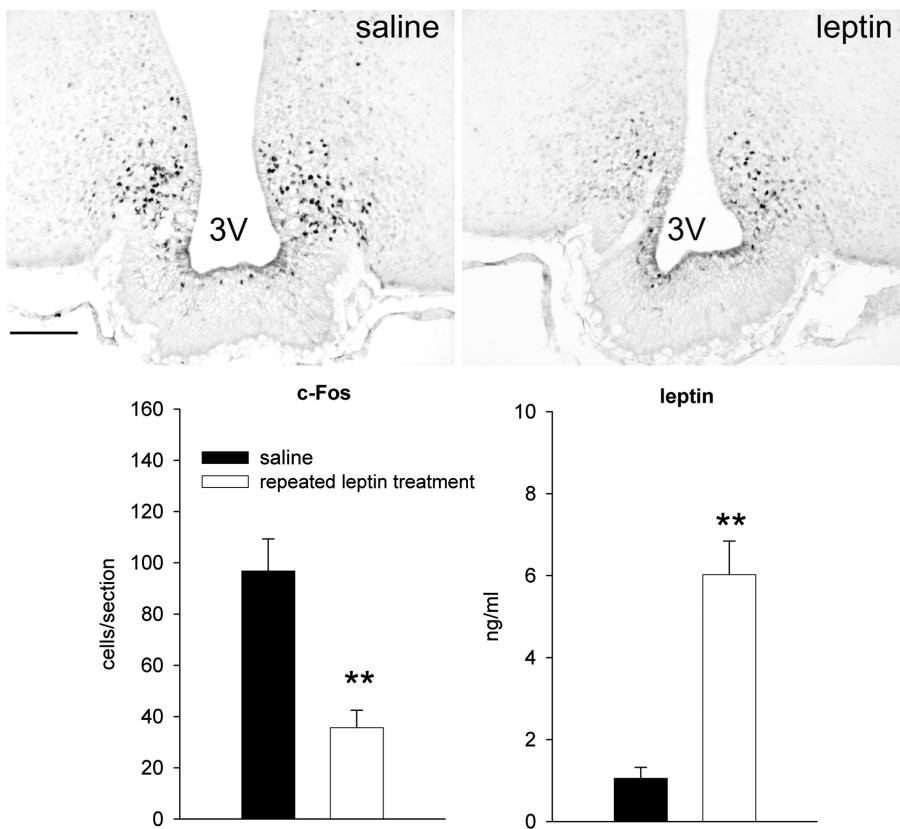


Figure 4: Effect of exogenous leptin-induced hyperleptinemia during fasting on arcuate nucleus (ARC) activation in lean mice. Representative ARC sections immunostained for c-Fos of young lean mice treated with saline or leptin every 3 h during a 14-h food-deprivation period. Leptin treatment significantly increased the leptin plasma concentration and attenuated the fasting-induced c-Fos expression in the ARC (** $P < 0.01$ for both effects). 3V: 3rd ventricle. Scale bar: 100 μ m. Published in Becskei C. et al., Am J Physiol Regul Integr Comp Physiol 2010;299:R632-R641.

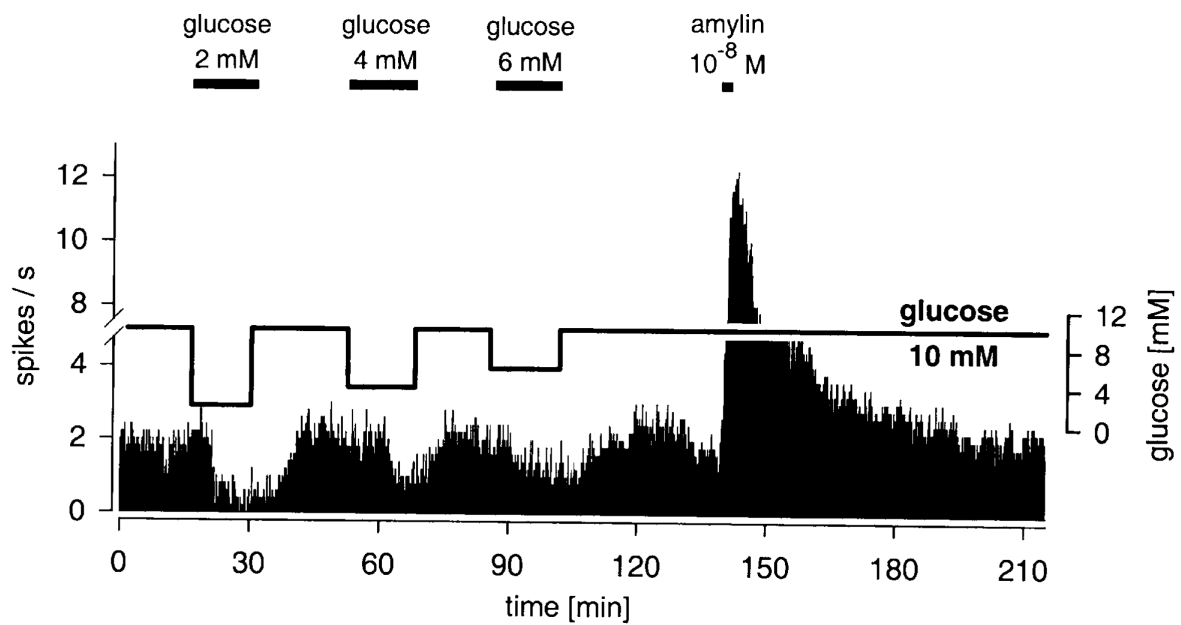


Figure 5: Co-sensitivity of an area postrema (AP) neuron to glucose and amylin. Decreasing the glucose concentration in the superfusion solution from the standard concentration of 10 mM to 2, 4 and 6 mM during the indicated time caused concentration-dependent decreases of the spontaneous discharge rate. Superfusion of amylin elicited a strong excitatory effect. Reproduced with permission (published in Riediger T, et al., *Neurosci Lett* 2002; 328:121-142).

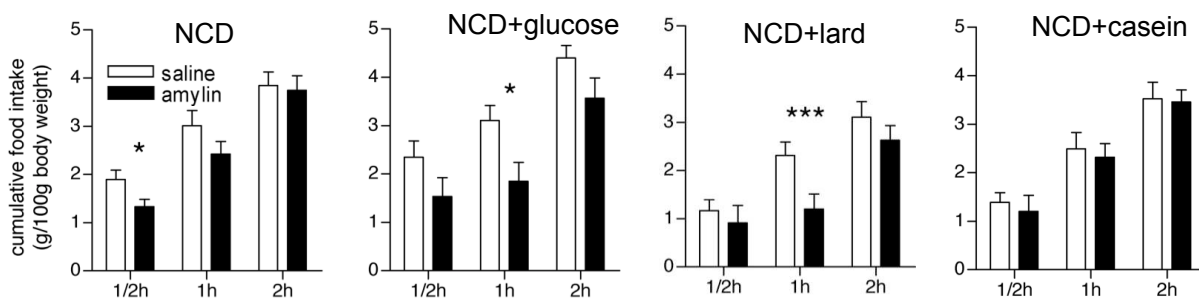


Figure 6: Effect of amylin (5 μ g/kg s.c.) on food intake in rats kept on different test diets (NCD = non-caloric diet, vanilla-flavored cellulose) for 24 h prior to injection. Bars represent group means \pm SEM (n = 12). * p < 0.05 and *** p < 0.001, significantly different from respective control (saline) group (paired Student t test). Modified and reproduced with permission (published in Michel et al., *Neuroendocrinology* 2007; 86:124-135).

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